09/076,115

Refine Search

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Term	Documents
SUPERSCRIPT	6130
SUPERSCRIPTS	1425
(1 AND SUPERSCRIPT).USPT,EPAB,JPAB,DWPI.	0
(L1 AND SUPERSCRIPT).USPT,EPAB,JPAB,DWPI.	0

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DATE: Friday, May 21, 2004 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ			
L2	L1 and superscript	0	L2
L1	5968784.pn.	2	L1

END OF SEARCH HISTORY

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US Patents Full-Text Database
US OCR Full-Text Database
EPO Abstracts Database
JPO Abstracts Database
Derwent World Patents Index
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Term: L11 and (mak$3 cDNA or produc$3 cDNA or prepar$3 cDNA or sysnthesiz$3 cDNA)

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ **Hit List** ◉ **Hit Count** ○ **Side by Side** ○ **Image**

Search Clear Interrupt

Search History

DATE: Friday, May 21, 2004 Printable Copy Create Case

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DB=USPT,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ			
L12	L11 and (mak$3 cDNA or produc$3 cDNA or prepar$3 cDNA or sysnthesiz$3 cDNA)	2	L12
L11	SuperScript same (benefit$1 or advantag$4)	71	L11
L10	SuperScript near5 (benefit$1 or advantag$4)	0	L10
L9	l4 and (useful or benefit$1 or advantag$4)	711	L9
L8	l5 and (benifit or advantig$4)	1	L8
L7	SuperScript near5 (benifit or advantig$4)	0	L7
L6	L5 and NotI	2	L6
L5	SuperScript near5 reverse transcriptase$1 near5 cDNA	127	L5
L4	SuperScript near5 reverse transcriptase$1	735	L4
L3	L2	0	L3
L2	L1 and superscript	0	L2
L1	5968784.pn.	2	L1

END OF SEARCH HISTORY

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=> s SuperScript(10a)stud####(10a)cDNA
L1            0 SUPERSCRIPT(10A) STUD####(10A) CDNA

=> s SuperScript (10a)cDNA
L2           40 SUPERSCRIPT (10A) CDNA

=> s l2 and stud####
L3           13 L2 AND STUD####

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4           12 DUP REM L3 (1 DUPLICATE REMOVED)

=> d l4 1-12 bib ab kwic

L4   ANSWER 1 OF 12  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN   2004:133932  BIOSIS
DN   PREV200400132158
TI   Hepcidin expression is down-regulated in alcohol-fed rats: A possible
     pathogenic factor in alcohol related hepatic siderosis.
AU   Cheung, Ting Kin [Reprint Author]; Fletcher, Linda [Reprint Author];
     Bridle, Kim; Murphy, Therese [Reprint Author]; Crawford, Darrell [Reprint
     Author]
CS   Princess Alexandra Hospital, Woolloongabba, QLD, Australia
SO   Hepatology, (October 2003) Vol. 38, No. 4 Suppl. 1, pp. 661A. print.
     Meeting Info.: 54th Annual Meeting of the American Association for the
     Study of Liver Diseases. Boston, MA, USA. October 24-28, 2003. American
     Association for the Study of Liver Diseases.
     ISSN: 0270-9139 (ISSN print).
DT   Conference; (Meeting)
     Conference; Abstract; (Meeting Abstract)
LA   English
ED   Entered STN: 10 Mar 2004
     Last Updated on STN: 10 Mar 2004
AB   Introduction: Both alcohol and excess iron in the liver can induce tissue
     damage and lead to fibrosis and ultimately cirrhosis.  Hepatic siderosis
     is often seen in patients with alcoholic liver disease.  The pathogenic
     mechanisms underlying this phenomenon have not been well characterized.
     Hepcidin, a recently discovered liver-derived circulating peptide, has
     been implicated in the homeostasis of iron metabolism and in the
     regulation of iron absorption.  Hepcidin is believed to be a negative
     regulator of intestinal iron absorption.  Divalent metal transporter 1
     (DMT1) and iron-regulated protein (IREG1) are the major iron transporters
     in duodenal enterocytes, with DMT1 responsible for apical uptake and IREG1
     for basolateral export of iron.  The effect of alcohol on the expression
     of hepcidin, DMT1 and IREG1 is not known.  Aim: To study the
     hepatic gene expression of hepcidin, DMT1 and IREG1 in a rat model of
     alcoholic liver disease.  Methods: Male Sprague Dawley rats (n=10) were
     pair-fed an alcoholic (Lieber-deCarli) liquid diet or a control liquid
     diet for 12 weeks.  Blood ethanol levels and serum AST were measured.
     Total liver RNA was extracted using Trizol(tm). cDNA was
     constructed using Superscript II(tm) following the
     manufacturer's instructions.  Quantitative (real-time) PCR was performed
     using SYBR green and specific primers for the hepcidin gene, DMT1 and
     IREG1-with GAPDH as the control gene.  Hepatic steatosis, inflammation and
     fibrosis were assessed histologically and hepatic iron concentration was
     measured colorimetrically.  Results: Blood ethanol levels in alcohol-fed
     rats were significantly higher than controls (229.2+-32.1 vs. 4.5+-0.4
     mg/dL; p<0.002).  Serum AST concentration was mildly increased in the
     alcohol-fed group but did not achieve statistical significance (101+-10.1
     vs. 87.8+-3.3 U/ml).  Histological examination revealed extensive
     steatosis in the alcohol-fed rats with minimal inflammation and fibrosis.
     Serum ferritin (656.6+-41.2 vs. 973.2+-186.2 mug/L) and hepatic iron
     concentrations (6.2+-0.8 vs. 6.8+-0.7 mumol/g dry weight) were not
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significantly different. Hepcidin mRNA expression was significantly decreased (7.4 fold reduction, p=0.009) in alcohol-fed rats compared to their pair-fed controls, however, hepatic DMT1 and IREG1 remained unchanged. Conclusion: Hepcidin expression is significantly decreased in the alcohol-fed rats. Altered hepcidin expression may be an important contributing factor to hepatic siderosis often seen in human alcoholic liver disease.

AB. . . export of iron. The effect of alcohol on the expression of hepcidin, DMT1 and IREG1 is not known. Aim: To **study** the hepatic gene expression of hepcidin, DMT1 and IREG1 in a rat model of alcoholic liver disease. Methods: Male Sprague. . . liquid diet for 12 weeks. Blood ethanol levels and serum AST were measured. Total liver RNA was extracted using Trizol(tm). **cDNA** was constructed using **Superscript** II(tm) following the manufacturer's instructions. Quantitative (real-time) PCR was performed using SYBR green and specific primers for the hepcidin gene,. . .

L4 ANSWER 2 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2003:402066 BIOSIS
DN PREV200300402066
TI Detection and partial cloning of a novel gene transcript from activated rat macrophages.
AU Baker, Brian C. [Reprint Author]; Chapman, Judith; Jadus, Martin; Williams, Christopher; Klasen, Maik; Acey, Roger A.
CS Chemistry and Biochemistry, California State University, 1250 Bellflower, Long Beach, CA, 90840, USA
yadirab@juno.com; microchixx@aol.com; jadus.martin@long-beach.va.gov; stlukemd@aol.com; maikklasen@hotmail.com; racey@csulb.edu
SO FASEB Journal, (March 2003) Vol. 17, No. 4-5, pp. Abstract No. 372.11. http://www.fasebj.org/. e-file.
Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. San Diego, CA, USA. April 11-15, 2003. FASEB.
ISSN: 0892-6638 (ISSN print).
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 3 Sep 2003
Last Updated on STN: 3 Sep 2003
AB Previous **studies** have shown that when T9 glioma tumor cells transfected with membrane bound macrophage colony stimulating factor (mM-CSF) are injected intra-cranially into rats, a macrophage cytotoxic response to the tumor is induced; 80% of the animals survived for at least 10 days. In addition, these animals develop an immunity to subsequent intracranial injections of parental T9 tumor cells. Our objective is to identify gene products that are induced in macrophages activated by mM-CSF. RNA from different temporal stages of MCSF activated macrophages was analyzed by differential display for ETS. Total RNA was converted to **cDNA** using **Superscript** II reverse transcriptase and anchored oligo dT as primer. The cDNA was then amplified using the same oligo dT (3'primer) and random decamers (5'primer). Analysis of the PCR products on urea-polyacryalamide gels revealed a number of differentially expressed transcripts. In one case, a prominent ETS (ca. 800 bp) is down regulated during the first 8 hours of activation. The ETS has significant homology to a Riken cDNA clone from a mouse mammary tumor. Northern blot analysis of macrophage RNA revealed the full-length transcript to be ca. 1600 bp.
AB Previous **studies** have shown that when T9 glioma tumor cells transfected with membrane bound macrophage colony stimulating factor (mM-CSF) are injected intra-cranially. . . from different temporal stages of MCSF activated macrophages was analyzed by differential display for ETS. Total RNA was converted to **cDNA** using **Superscript** II reverse transcriptase and anchored oligo dT as primer. The cDNA was then amplified using the same oligo dT (3'primer). . .

L4 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:588647 CAPLUS
DN 139:112759
TI Protein and cDNA sequences of a human glial-derived neurotrophic factor and therapeutic use for nervous system diseases
IN Zhou, Shoushan; Zheng, Zanshun; Fang, Haizhou; Chen, Yong; Jiang, Ruofeng; Zhu, Aitang; Zhang, Qi; Gan, Shuyan; Lan, Xuan
PA Zhuhai Yisheng Biopharmaceuticals Co., Ltd., Peop. Rep. China
SO Faming Zhuanli Shenqing Gongkai Shuomingshu, 28 pp.
CODEN: CNXXEV
DT Patent
LA Chinese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	CN 1364812	A	20020821	CN 2001-107450	20010111
PRAI	CN 2001-107450		20010111		

AB The invention provides the DNA sequence or its DNA fragments and their encoded amino acid sequences of human glial-derived neurotrophic factor (GDNF) cloned from human glioma cell line C6 or synthesized by solid-phase synthesis method. The invention relates to the construction of the expression vector, the expression of GDNF in E.coli, yeast, and CHO cells, and separation and purification of GDNF from the cultured products of the above genetically engineered bacteria or CHO cells. The invention also relates to the application of the expressed GDNF in preparing the medical composition (composed of GDNF, natural ganglioside or its derivative, and/or mycose or hyaluronic acid) for treating nervous system disease, insanity, etc.
IT Carbohydrates, biological **studies**
Gangliosides
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(protein and cDNA sequences of human glial-derived neurotrophic factor and therapeutic use for nervous system diseases)
IT 9068-38-6, Reverse transcriptase
RL: RGT (Reagent); RACT (Reactant or reagent)
(**superscript**; protein and **cDNA** sequences of human glial-derived neurotrophic factor and therapeutic use for nervous system diseases)

L4 ANSWER 4 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2003:367798 BIOSIS
DN PREV200300367798
TI Comparison of Differentially Expressed Genes between AML and RAEBt Using Two Different Softwares for Data Analysis.
AU Bellido, Mar [Reprint Author]; Stirewalt, Derek L. [Reprint Author]; Pogosova, Era L. [Reprint Author]; Kussick, Steve [Reprint Author]; Sala, Olga [Reprint Author]; Deeg, Joachim [Reprint Author]; Radich, Jerald P. [Reprint Author]
CS Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
SO Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 4314. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
DT Conference; (Meeting)
Conference; (Meeting Poster)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 13 Aug 2003
Last Updated on STN: 13 Aug 2003
AB Aim : Microarray analysis using oligonucleotide arrays was performed to identify differentially expressed genes between de novo AML and RAEBt. To determine if reproducible genetic profiles can be consistently obtained,

two different softwares were used for data analysis. Methods : Eight samples from 4 patients with de novo AML and 4 patients with RAEBt were **studied**. The samples were processed as described in the Affymetrix GeneChip Expression Analysis Technical Manual using 5 ug of total RNA. Briefly, total RNA was converted to first-strand **cDNA** using **Superscript** II reverse transcriptase primed by an oligomer that incorporated the T7 promoter. After the synthesis of the second-strand cDNA, an in vitro transcription for linear amplification of each transcript was done using biotinylated nucleotides. The cRNA products were fragmented, heated and hybridized to the human array HG-U133A chip (Affymetrix) which contains 22577 human genes. The microarrays were washed and stained and fluorescence was amplified and collected in a confocal scanner. Data analysis : The software developed for Affymetrix (M.A.S 5.0) provided the initial data to perform a global comparison between the experiments. Probe pairs were scored positive or negative following this formula: R = (PM-MM)/(PM +MM) and global scaling was applied to allow comparison of gene signals across multiple microarrays. The default discrimination threshold (tau = 0.015) was used to assign a call of absent, present or marginal for each assayed gene, and a p-value was calculated to reflect confidence in the detection call. This single array analysis generated the expression values for each gene and the file with the intensity of each probe cell (.CEL). We applied 2 different softwares, geneplus v1.2 (Enodar BioLogic Corporation, Seattle) based on regression analysis and dCHIP v1.1 (Harvard University, Boston), which uses the model-based expression approach to analyze multiple arrays. Results : Data analysis resulted in different sets of genes, 70 using geneplus and 40 using dCHIP. Proteins belonging to the zinc-finger protein family, bone marrow matrix, transcription factors and to clones with unknown genes constituted most of the differentially expressed genes. An exploratory clustering using both sets of genes allowed to differentiate AML from RAEBt. Comments : Data analysis of oligonucleotide arrays performed with different measures of transcript amount can result in different results, even although the same initial dataset is used.

AB. . . for data analysis. Methods : Eight samples from 4 patients with de novo AML and 4 patients with RAEBt were **studied**. The samples were processed as described in the Affymetrix GeneChip Expression Analysis Technical Manual using 5 ug of total RNA. Briefly, total RNA was converted to first-strand **cDNA** using **Superscript** II reverse transcriptase primed by an oligomer that incorporated the T7 promoter. After the synthesis of the second-strand cDNA, an. . .

L4 ANSWER 5 OF 12 MEDLINE on STN DUPLICATE 1
AN 2002247902 MEDLINE
DN PubMed ID: 11986703
TI Expression and drug resistance of human MGMT gene in hemopoietic cells mediated by bicistronic retroviral vector.
AU Wang J; Xia X; Chen Z; Ruan C G
CS Jiangsu Institute of Hematology, First Affiliated Hospital of Suzhou Medical College, Suzhou 215006, China.
SO Zhonghua shi yan he lin chuang bing du xue za zhi = Zhonghua shiyan he linchuang bingduxue zazhi = Chinese journal of experimental and clinical virology, (2001 Sep) 15 (3) 265-8.
Journal code: 9602873. ISSN: 1003-9279.
CY China
DT Journal; Article; (JOURNAL ARTICLE)
LA Chinese
FS Priority Journals
EM 200302
ED Entered STN: 20020503
Last Updated on STN: 20030227
Entered Medline: 20030226
AB OBJECTIVE: To increase myeloid progenitors resistance to chemotherapy and prevent myelosuppression caused by alkylating agents. METHODS: Total cellular RNA was extracted from human liver and **cDNA** was

synthesized by **superscript** reverse transcriptase, a polymerase chain reaction(PCR) was conducted. We obtained a full length cDNA fragment encoding human alkyguarine-DNA-alhyltransferase(MGMT). The PCR product was cloned into pGEMT-T vector and further subcloned into G1Na retrovirus expression vector. Then the recombinant plasmid was transduced into the packaging cell lines GP+E86 and PA317 by lipofect AMINE. RESULTS: By using the medium containing BCNU for cloning selection and ping-ponging supernatant infection between ecotropic produced clone and amphotropic producer clone, we obtained high titer amphotropic PA317 producer clone with the highest titer up to 8.6-10 CFU/ml. Human hematopoietic cells were infected repeatedly with this high titer virus under stimulation of hemopoietic growth factors IL-3, IL-6 and SCF. PCR, RT-PCR, Southern blot, Western blot and MTT analyses showed that MGMT gene has integrated into the genomic DNA of human hemopoietic cells and expressed efficiently. CONCLUSIONS: This **study** provides a foundation for application of gene therapy to tumor clinical trial.

AB . . . resistance to chemotherapy and prevent myelosuppression caused by alkylating agents. METHODS: Total cellular RNA was extracted from human liver and **cDNA** was synthesized by **superscript** reverse transcriptase, a polymerase chain reaction(PCR) was conducted. We obtained a full length cDNA fragment encoding human alkyguarine-DNA-alhyltransferase(MGMT). The PCR. . . analyses showed that MGMT gene has integrated into the genomic DNA of human hemopoietic cells and expressed efficiently. CONCLUSIONS: This **study** provides a foundation for application of gene therapy to tumor clinical trial.

L4 ANSWER 6 OF 12 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

AN 2001030872 EMBASE

TI Toll-like receptor 4: The missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components.

AU Laflamme N.; Rivest S.

CS S. Rivest, Lab. of Molecular Endocrinology, CHUL Research Center, Laval University, 2705, boul. Laurier, Quebec, Que. GIV 4G2, Canada. Serge.Rivest@crchul.ulaval.ca

SO FASEB Journal, (2001) 15/1 (155-163).
Refs: 19
ISSN: 0892-6638 CODEN: FAJOEC

CY United States

DT Journal; Article

FS 026 Immunology, Serology and Transplantation

LA English

SL English

AB The recent characterization of human homologues of Toll may be the missing link for the transduction events leading to NF-κB activity and proinflammatory gene transcription during innate immune response. Indeed, CD14 is not thought to participate directly in the cell signaling, but rather one or more of the mammalian Toll-like receptors (TLRs) acts in concert with the lipopolysaccharide (LPS) receptor to discriminate between microbial pathogens or their products and initiate transmembrane signaling. Mammalian cells may express as many as 10 distinct TLRs, although the importance of TLR4 in response to gramnegative bacteria and LPS is now supported by the fact that TLR4-mutated mice are LPS resistant. We investigated the expression of TLR4 across the rat brain under basal conditions and in response to systemic LPS and IL-1β injection. We first cloned the rat TLR4 cDNA via RNA isolation and polymerase chain reaction (PCR) amplification with a proofreading polymerase. Total RNA was isolated from the rat liver tissue using Tri-Reagent and reverse transcribed into **cDNA** using **Superscript** II reverse transcriptase and an oligonucleotide primer with a degenerate 3' end of sequence 5'-T12(GAC)N-3'. Positive hybridization signal was found in the leptomeninges, choroid plexus (chp), subfornical organ, organum vasculosum of the lamina terminalis, median eminence, and area postrema. Scattered

small cells also displayed a convincing hybridization signal within the brain parenchyma. Few well-defined nuclei exhibited positive TLR4 transcript: the supramamillary nucleus, cochlear nucleus, and the lateral reticular nucleus. The circumventricular organs, the leptomeninges, and chp also exhibited constitutive expression of the LPS receptor mCD14. In contrast to the strong up-regulation of the gene encoding mCD14 during endotoxemia, neither LPS nor IL-1β caused a convincing increase in the TLR4 mRNA levels across the CNS. A down-regulation of the gene encoding TLR4 was found in the cerebral tissue of immunechallenged animals. The constitutive expression of both mCD14 and TLR4 may explain the innate immune response in the brain, which originates from the structures devoid of blood-brain barrier in presence of circulating LPS.

AB . . . amplification with a proofreading polymerase. Total RNA was isolated from the rat liver tissue using Tri-Reagent and reverse transcribed into **cDNA** using **Superscript** II reverse transcriptase and an oligonucleotide primer with a degenerate 3' end of sequence 5'-T12(GAC)N-3'. Positive hybridization signal was found. . .

CT Medical Descriptors:
*brain
*immunity
*bacterial cell wall
*Gram negative bacterium
*nucleotide sequence
signal transduction
inflammation
polymerase chain reaction
blood brain barrier
in situ hybridization
microglia
septic shock
cytokine release
molecular cloning
RNA probe
nonhuman
male
rat
animal experiment
animal model
controlled study
animal tissue
article
priority journal
*toll like receptor 4: EC, endogenous compound
*immunoglobulin enhancer binding protein: EC, endogenous compound
*bacterium lipopolysaccharide: EC, endogenous compound
CD14. . .

L4 ANSWER 7 OF 12 MEDLINE on STN
AN 2001351183 MEDLINE
DN PubMed ID: 11414441
TI A novel method for the preparation of large cDNA fragments from dengue-3 RNA genome by long RT-PCR amplification.
AU Attatippaholkun W H; Attatippaholkun M K; Nisalak A; Vaughn D W; Innis B L
CS Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.
SO Southeast Asian journal of tropical medicine and public health, (2000) 31 Suppl 1 126-33.
Journal code: 0266303. ISSN: 0125-1562.
CY Thailand
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200110
ED Entered STN: 20011029

Last Updated on STN: 20011029
Entered Medline: 20011025

AB For many years, dengue viruses were among the most difficult flaviviruses to isolate and to identify, but technical advances in the past 20 years have facilitated this process. Dengue viruses are usually recovered from specimens by the infection of mosquito-cell cultures. The virus may be passaged several times in cell cultures until a sufficient infectivity titer is attained. The viral nucleocapsid consists of capsid protein and an RNA genome. The dengue genome is a single stranded messenger (positive) sense RNA of approximately 11 kb in length. The isolation of dengue genomic RNA from various sources requires precautions to avoid RNases. RNases are released during cell disruption, and their activity must be inhibited as quickly as possible by using guanidinium thiocyanate in the presence of 2-mercaptoethanol. There has recently been a revolution in molecular biology with the development of the powerful reverse transcriptase (RT) and polymerase chain reaction (PCR) technology. Advanced **studies** on RT technique lead to much further improvement of the reverse transcriptase enzyme by genetic engineering. The Superscript II RNase H- RT (GIBCO BRL, USA) is genetically engineered DNA polymerase that synthesizes a complementary DNA strand from single-stranded RNA. DNA or an RNA-DNA hybrid. This enzyme is produced from a cloned M-MLV RT gene constructed by the introduction of point mutation in the RNase H active center. The selective mutations within the RNase H domain maintain full polymerase activity. This structural modification eliminates degradation of RNA molecules during the first strand cDNA synthesis. The combination of thermostable DNA polymerase with and without proofreading activity (3'-exonuclease activity), improved buffer conditions and thermal cycling profiles overcome the length limitation of PCR. On the basis of these findings, we have developed a long RT-PCR system for preparing large **cDNA** fragments of dengue 3 virus (H-87) by using the **Superscript** II RNase H- RT for reverse transcription and a mixture of Taq and Pwo DNA polymerases for PCR. Three large cDNA fragments covered the full genomic RNA from the 5'-end to the 3'-end of dengue-3 virus (H-87; 10,696 bps) could be successfully prepared as the lengths of 2.437 bps, 3,980 bps and 4,337 bps respectively. The ability of our developed long RT-PCR will bring speed and simplicity to genomic mapping and sequencing and facilitate **studies** in molecular genetics of dengue viruses.

AB . . . revolution in molecular biology with the development of the powerful reverse transcriptase (RT) and polymerase chain reaction (PCR) technology. Advanced **studies** on RT technique lead to much further improvement of the reverse transcriptase enzyme by genetic engineering. The Superscript II RNase. . . length limitation of PCR. On the basis of these findings, we have developed a long RT-PCR system for preparing large **cDNA** fragments of dengue 3 virus (H-87) by using the **Superscript** II RNase H- RT for reverse transcription and a mixture of Taq and Pwo DNA polymerases for PCR. Three large. . . respectively. The ability of our developed long RT-PCR will bring speed and simplicity to genomic mapping and sequencing and facilitate **studies** in molecular genetics of dengue viruses.

L4 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:223671 BIOSIS
DN PREV200200223671
TI The effect of low sodium diet on loop of Henle bicarbonate transport and medullary thick ascending limb NHE-3 gene expression.
AU Capasso, Giovambattista [Reprint author]; Rizzo, Maria [Reprint author]; Deligatti, Angela [Reprint author]; Ferrara, Daniele [Reprint author]
CS Nephrology, Faculty of Medicine, Second University of Naples, Naples, Italy
SO Journal of the American Society of Nephrology, (September, 2000) Vol. 11, No. Program and Abstract Issue, pp. 2A. print.
Meeting Info.: 33rd Annual Meeting of the American Society of Nephrology and the 2000 Renal Week. Toronto, Ontario, Canada. October 10-16, 2000.

American Society of Nephrology.
CODEN: JASNEU. ISSN: 1046-6673.

DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)

LA English

ED Entered STN: 3 Apr 2002
Last Updated on STN: 3 Apr 2002

AB It has been reported that sodium intake may affect renal tubular acidification. Previous data have also demonstrated that most of the bicarbonate transport along the loop of Henle (LOH) is localised at the level of the medullary thick ascending limb (mTAL) and it is mainly dependent from the presence of luminal Na+-H+ exchanger (NHE-3). In the present **study** we have investigated the effect of low sodium diet (LSD) on: 1. bicarbonate transport along the LOH; 2. NHE-3 gene expression at the level of mTALs. The experiments were performed on two groups of rats: 1. control animals; 2. rats fed for fourteen days with LSD. To measure net bicarbonate reabsorption (JHCO3) superficial loops were perfused from late proximal to early distal tubules by in vivo micropuncture. Perfusate was an end-like proximal solution containing 3H-methoxy-inulin. Total (CO2) in the collected fluid was measured by microcalorimetry. In the presence of similar LOH perfusion rate (39.1+-0.9 vs 38.7+-0.9 nl/min), JHCO3 was 302+-26 pmol/min (n=20) (mean+-ES) in control animals and 295+-23 pmol/min (n=22, p>0.05) in rats under LSD. For the molecular biology experiments total RNA was extracted from mTALs, identified and microdissected from collagenase treated kidneys. **cDNA** was synthesised from RNA using **SuperScript** RT Rnase, H-reverse Transcriptase and oligo(dT) 12-18 primers; each reaction was performed in parallel with an otherwise identical reaction that contained no Reverse Transcriptase. NHE-3 mRNA abundance was quantified by a competitive PCR using an internal standard of cDNA which differed from the wild-type NHE-3 by a deletion of 76 bp. The PCR was performed starting from the same amount of total RNA. NHE-3 mRNA abundance was 0.133+-0.016 amol/ng total RNA in controls (n=5) and it decreased to 0.068+-0.007 amol/ng total RNA in rats under LSD (n=5, p<0.005). These data demonstrate that LSD does not affect LOH bicarbonate transport, but it decreases NHE-3 gene expression along mTAL. Therefore the preservation of unchanged proton secretion along the mTAL may be dependent from other molecular mechanism(s).

AB. . . thick ascending limb (mTAL) and it is mainly dependent from the presence of luminal Na+-H+ exchanger (NHE-3). In the present **study** we have investigated the effect of low sodium diet (LSD) on: 1. bicarbonate transport along the LOH; 2. NHE-3 gene. . . under LSD. For the molecular biology experiments total RNA was extracted from mTALs, identified and microdissected from collagenase treated kidneys. **cDNA** was synthesised from RNA using **SuperScript** RT Rnase, H-reverse Transcriptase and oligo(dT) 12-18 primers; each reaction was performed in parallel with an otherwise identical reaction that. . .

L4 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

AN 2001:87416 BIOSIS

DN PREV200100087416

TI Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating Gram-negative bacterial cell wall components.

AU Laflamme, N. [Reprint author]; Rivest, S.

CS CHUL Research Center, Sainte-Foy, PQ, Canada

SO Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-242.17. print.
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 04-09, 2000. Society for Neuroscience.
ISSN: 0190-5295.

DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LA English
ED Entered STN: 14 Feb 2001
Last Updated on STN: 12 Feb 2002
AB The recent characterization of human homologues of Toll may be the missing link for the transduction events leading to NF-kappaB activity and proinflammatory gene transcription during innate immune response. Indeed, CD14 is not thought to participate directly in the cell signaling, but rather one or more of the mammalian Toll-like receptors (TLRs) acts in concert with the LPS receptor to discriminate between microbial pathogens or their products and initiate transmembrane signaling. Mammalian cells may express as many as ten distinct TLRs, although the importance of TLR4 in response to Gram-negative bacteria and LPS is now supported by the fact that TLR4-mutated mice are LPS resistant. The present **study** investigated the expression of TLR4 across the rat brain under basal conditions and in response to systemic LPS and IL-1beta injection. We first cloned the rat TLR4 cDNA via RNA isolation and PCR amplification with a proof-reading polymerase. Total RNA was isolated from rat liver tissue using Tri-Reagent and reverse transcribed into **cDNA** using **Superscript** II reverse transcriptase and an oligonucleotide primer with a degenerate 3' end of sequence 5'-T12(GAC)N-3'. Positive hybridization signal was found in the leptomeninges, choroid plexus (chp), subfornical organ, organum vasculosum of the lamina terminalis, median eminence and area postrema. Scattered small cells also displayed a convincing hybridization signal within the brain parenchyma, although the intensity was quite variable among animals. Few neuronal structures exhibited positive TLR4 transcript, namely the supramammillary nucleus, cochlear nucleus and caudal ventrolateral medulla. Of interest is the fact the circumventricular organs, the leptomeninges and chp also exhibited constitutive expression of the LPS receptor mCD14. In contrast to the strong upregulation of the gene encoding mCD14 during endotoxemia, neither LPS nor IL-1beta caused a convincing increase in the TLR4 mRNA levels across the CNS. The constitutive expression of both mCD14 and TLR4 may explain the innate immune response in the brain, which originates from the structures devoid of blood brain barrier in presence of circulating LPS.
AB. . . response to Gram-negative bacteria and LPS is now supported by the fact that TLR4-mutated mice are LPS resistant. The present **study** investigated the expression of TLR4 across the rat brain under basal conditions and in response to systemic LPS and IL-1beta. . . PCR amplification with a proof-reading polymerase. Total RNA was isolated from rat liver tissue using Tri-Reagent and reverse transcribed into **cDNA** using **Superscript** II reverse transcriptase and an oligonucleotide primer with a degenerate 3' end of sequence 5'-T12(GAC)N-3'. Positive hybridization signal was found. . .

L4 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:761906 CAPLUS
DN 130:11283
TI Production and purification of nucleic acid molecules using affinity-labeled primer-adapter molecules
IN Gruber, Christian E.; Jessee, Joel A.
PA Life Technologies, Inc., USA
SO PCT Int. Appl., 50 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9851699	A1	19981119	WO 1998-US9586	19980512
	W: JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	EP 981535	A1	20000301	EP 1998-922202	19980512

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 2002502237 T2 20020122 JP 1998-549380 19980512

PRAI US 1997-46219P P 19970512

WO 1998-US9586 W 19980512

AB The present invention is directed to methods for the production and isolation of nucleic acid mols. In particular, the invention concerns isolation of mRNA mols. and the production and isolation of nucleic acid mols. (e.g., cDNA mols. or libraries), which may be single- or double-stranded. Addnl., the invention concerns selection and isolation of particular nucleic acid mols. of interest from a sample which may contain a population of mols. Specifically, the invention concerns affinity-labeled primer-adapter mols. which allow improved isolation and production of such nucleic acid mols., increasing both product recovery and speed of isolation. A mRNA or poly(A)+ RNA mols is mixed with a polymerase and/or reverse transcriptase and a primer-adapter nucleic acid mol., wherein the primer-adaptor comprises one or more ligand mols. and one or more cleavage sites (for endonuclease or restriction endonuclease). The primer-adaptor may be designed to hybridize to any portion of the template. Upon incubation under appropriate conditions, a first nucleic acid mol. (e.g., a single-stranded cDNA) complementary to all or a portion of the template is made, which contains the primer-adaptor and thereby facilitates isolation of the first nucleic acid mol. and/or any nucleic acid mol. hybridized to the first nucleic acid. Such isolation may be accomplished by ligand-hapten interactions, where the hapten is bound to a solid support. Multiple synthesis with primer-adaptor mols. may result in a synthesized nucleic acid mol. having more than one primer-adaptor. The present invention is particularly suited for the rapid production and isolation of cDNA libraries from small amts. (as little of 5 ng) of input poly(A)+ RNA or mRNA in a high-throughput manner. About 3-4-fold greater yield of **cDNA** is achieved by the present invention than with the **SUPERSCRIPT** Plasmid System, with approx. equivalent transformation efficiencies and average insert sizes.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB The present invention is directed to methods for the production and isolation of nucleic acid mols. In particular, the invention concerns isolation of mRNA mols. and the production and isolation of nucleic acid mols. (e.g., cDNA mols. or libraries), which may be single- or double-stranded. Addnl., the invention concerns selection and isolation of particular nucleic acid mols. of interest from a sample which may contain a population of mols. Specifically, the invention concerns affinity-labeled primer-adapter mols. which allow improved isolation and production of such nucleic acid mols., increasing both product recovery and speed of isolation. A mRNA or poly(A)+ RNA mols is mixed with a polymerase and/or reverse transcriptase and a primer-adapter nucleic acid mol., wherein the primer-adaptor comprises one or more ligand mols. and one or more cleavage sites (for endonuclease or restriction endonuclease). The primer-adaptor may be designed to hybridize to any portion of the template. Upon incubation under appropriate conditions, a first nucleic acid mol. (e.g., a single-stranded cDNA) complementary to all or a portion of the template is made, which contains the primer-adaptor and thereby facilitates isolation of the first nucleic acid mol. and/or any nucleic acid mol. hybridized to the first nucleic acid. Such isolation may be accomplished by ligand-hapten interactions, where the hapten is bound to a solid support. Multiple synthesis with primer-adaptor mols. may result in a synthesized nucleic acid mol. having more than one primer-adaptor. The present invention is particularly suited for the rapid production and isolation of cDNA libraries from small amts. (as little of 5 ng) of input poly(A)+ RNA or mRNA in a high-throughput manner. About 3-4-fold greater yield of **cDNA** is achieved by the present invention than with the **SUPERSCRIPT** Plasmid System, with approx. equivalent transformation efficiencies and average insert sizes.

IT Ankyrins

Antibodies
Antigens
Avidins
CD4 (antigen)
Collagens, biological **studies**
Colony stimulating factor receptors
Cytokine receptors
Cytokines
Enzymes, biological **studies**
Fibrinogens
Fibronectins
Glycophorins
Growth factors, animal
Immunoglobulin receptors
Insulin receptors
Interleukins
LFA-1 (antigen)
Laminins
Lipopolysaccharides
Spectrins
Transferrin receptors
Transferrins
Vitronectin
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(ligand mol. for affinity labeling; production and purification of nucleic acid
mols. using affinity-labeled primer-adapter mols.)

IT 58-85-5, Biotin 1404-26-8, Polymyxin B 7439-89-6, Iron, biological **studies** 9001-29-0, Blood-coagulation factor X 9004-10-8, Insulin, biological **studies** 9013-20-1, Streptavidin 20074-52-6, biological **studies** 62683-29-8, Colony-stimulating factor 80804-53-1, Complement C3bi
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(ligand mol. for affinity labeling; production and purification of nucleic acid
mols. using affinity-labeled primer-adapter mols.)

L4 ANSWER 11 OF 12 EMBASE
on STN
AN 1998127821 EMBASE
TI A competitive reverse transcription-PCR to **study** apolipoprotein ε gene expression.
AU Rexin M.; Feussner G.
CS G. Feussner, Med. Universitatsklinik Heidelberg, Abteilung Inhere Medizin I, Endokrinologie und Stoffwechsel, Bergheimer Strasse 58, D-69115 Heidelberg, Germany. uk691jd@genius.embnet.dkfz-heidelberg.de
SO Clinical Chemistry, (1998) 44/4 (773-778).
Refs: 31
ISSN: 0009-9147 CODEN: CLCHAU
CY United States
DT Journal; Article
FS 022 Human Genetics
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LA English
SL English
AB We developed a rapid and simple competitive reverse transcription-polymerase chain reaction for the quantification of apoε mRNA in human monocyte-derived macrophages. The method was applied, and its reliability was shown in patients with the familial lipoprotein disorder, type III hyperlipoproteinemia. Type III hyperlipoproteinemic patients express markedly higher concentrations of apoε mRNA when compared

with healthy controls. Patients with this disease are usually (>90%) homozygous for a receptor binding-defective isoform of apolipoprotein apo E (apo E2). The higher expression of apoε mRNA in the patients could, therefore, be a physiological mechanism to compensate for functionally defective apo E. The developed procedure might be valuable in assessment of apoε gene expression in human disease.

TI A competitive reverse transcription-PCR to **study** apolipoprotein ε gene expression.

CT Medical Descriptors:
*hyperlipoproteinemia: DI, diagnosis
reversed phase high performance liquid chromatography
gene expression
reliability
receptor binding
human
male
female
clinical article
controlled study
human cell
adult
article
*apolipoprotein e: EC, endogenous compound
messenger rna: EC, endogenous compound

NP **(1) Superscript Preamplification System for First Strand cDNA Synthesis**; (2) Minicycler

L4 ANSWER 12 OF 12 MEDLINE on STN
AN 97193806 MEDLINE
DN PubMed ID: 9041391
TI A one-tube method of reverse transcription-PCR to efficiently amplify a 3-kilobase region from the RNA polymerase gene to the poly(A) tail of small round-structured viruses (Norwalk-like viruses).
AU Ando T; Monroe S S; Noel J S; Glass R I
CS Viral Gastroenteritis Section, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA.. txa5@CIDDVD1.em.cdc.gov
SO Journal of clinical microbiology, (1997 Mar) 35 (3) 570-7. Journal code: 7505564. ISSN: 0095-1137.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199706
ED Entered STN: 19970612
Last Updated on STN: 19980206
Entered Medline: 19970602

copy the reference

AB Amplification of a 3-kb genome region from the RNA polymerase gene to the 3' poly(A) tail of small round-structured virus (SRSV) by reverse transcription-PCR (RT-PCR) has been difficult to achieve because of a stable secondary structure in a region between the RNA polymerase gene and the 5' end of the second open reading frame. We have developed a one-tube RT-PCR method to efficiently amplify this region. The method comprises three procedures: purification of poly(A)+ RNA from a starting RNA solution by oligo(dT)30 covalently linked to latex particles, buffer exchange, and continuous RT and PCR in a single tube containing all reaction components. The key elements of this method are (i) first-strand **cDNA** synthesis with the **Superscript** II version of RNase H- Moloney murine leukemia virus reverse transcriptase at 50 degrees C for 10 min by using the RNA-oligo(dT)30 hybrid on the latex particles as the template and primer, and (ii) PCR by Taq and Pwo DNA polymerases mixed together with a mixture of 12 phased oligo(dT)25 antisense primers. The detection threshold of the one-tube RT-PCR method was as little as 0.2 ng of the crude RNA used as the source of the template. Using this method,

we obtained 3-kb products from 24 SRSV strains previously characterized into four genetic groups. These included 5 P1-A, 4 P1-B, 5 P2-A, and 10 P2-B strains. Because SRSVs have not yet been cultivated in vitro, this novel method should facilitate molecular characterization of SRSVs to provide a firm scientific foundation for improvements and refinements of SRSV diagnostics.

AB . . . RT and PCR in a single tube containing all reaction components. The key elements of this method are (i) first-strand **cDNA** synthesis with the **Superscript** II version of RNase H- Moloney murine leukemia virus reverse transcriptase at 50 degrees C for 10 min by using. . .

CT . . . Human
Base Sequence
Caliciviridae Infections: DI, diagnosis
Caliciviridae Infections: VI, virology
DNA Primers: GE, genetics
*DNA-Directed RNA Polymerases: GE, genetics
Evaluation Studies
Gastroenteritis: DI, diagnosis
Gastroenteritis: VI, virology
*Genes, Viral
Moloney murine leukemia virus: EN, enzymology
Norwalk virus: EN, enzymology
. . .

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